

Attachment A

Darbepoetin in 5/6 nephrectomy and effect on EPC

Low-dose darbepoetin treatment has beneficial effect on EPC's in the 5/6 nephrectomy model

Introduction

We have previously shown, that low-dose darbepoetin has a beneficial effect on survival and kidney histology in a 5/6 nephrectomy model. We now analysed, if darbepoetin will increase the number of proliferating EPC's in the blood circulation.

Animal model

The Animal Care Committee of Lower Saxony approved the study. We studied 20 Sprague-Dawley rats (Charles River Wiga GmbH, Sutzfeld, Germany) weighing between 250 and 300 g. We performed in the rats of Group 2 and 3 selective ligations of the extrarenal renal artery branches of the left renal artery under ketamine and xylazine anesthesia to obtain a two-thirds renal infarction. The opposite kidney was then removed. Group 1 represents a healthy control group. Before and after the operation the rats received a weekly injection of 0.9 % NaCl (Placebo) or 0.1 µg darbepoetin/kg body weight.

	Group 1	Group 2	Group 3
Name	control	Nephrectomy control	nephrectomy EPO
Disease	No	renal insufficiency	renal insufficiency
Substance	Placebo	Placebo	0.1 µg/kg body weight
Number	4	8	8

Hematocrit

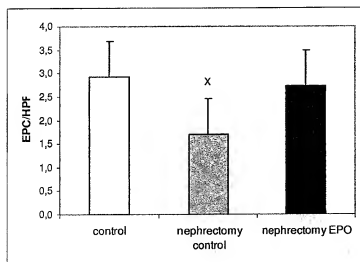
We assessed hematocrit with a vet scil abc (SCIL, Germany) at baseline and after 3 weeks.

The hematocrit was similar in all three groups at baseline. Three weeks after operation the HCT was slightly reduced in group 2 and 3. No difference was noted between the two groups.

Endothelial progenitor cells

Three weeks after the nephrectomy peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. Four days after EPC culture on rat vitronectin plus 0.5% gelatin (0.5×10^6 cells/well of a 24-well plate), EPCs were assayed by costaining with acetylated LDL (acLDL)/DiI (Biomedical Technologies) and fluorescein isothiocyanate (FITC)-conjugated BS-1 lectin (Vector). Double-positive cells per high-power field (HPF) ($\times 100$) were counted.

Control rats had significantly fewer proliferating EPCs after nephrectomy than healthy control rats. After 3 weeks the number of proliferating EPC's were significantly higher in the darbepoetin treated group.



x: $p < 0.05$ vs. control and nephrectomy EPO